Development of an LC-MS/MS method for **GSK3389404**, a GalNAc conjugated oligonucleotide and it's non-conjugated metabolite in pre-clinical and clinical studies – challenges and strategies

Alexandra Bushby, Labcorp UK

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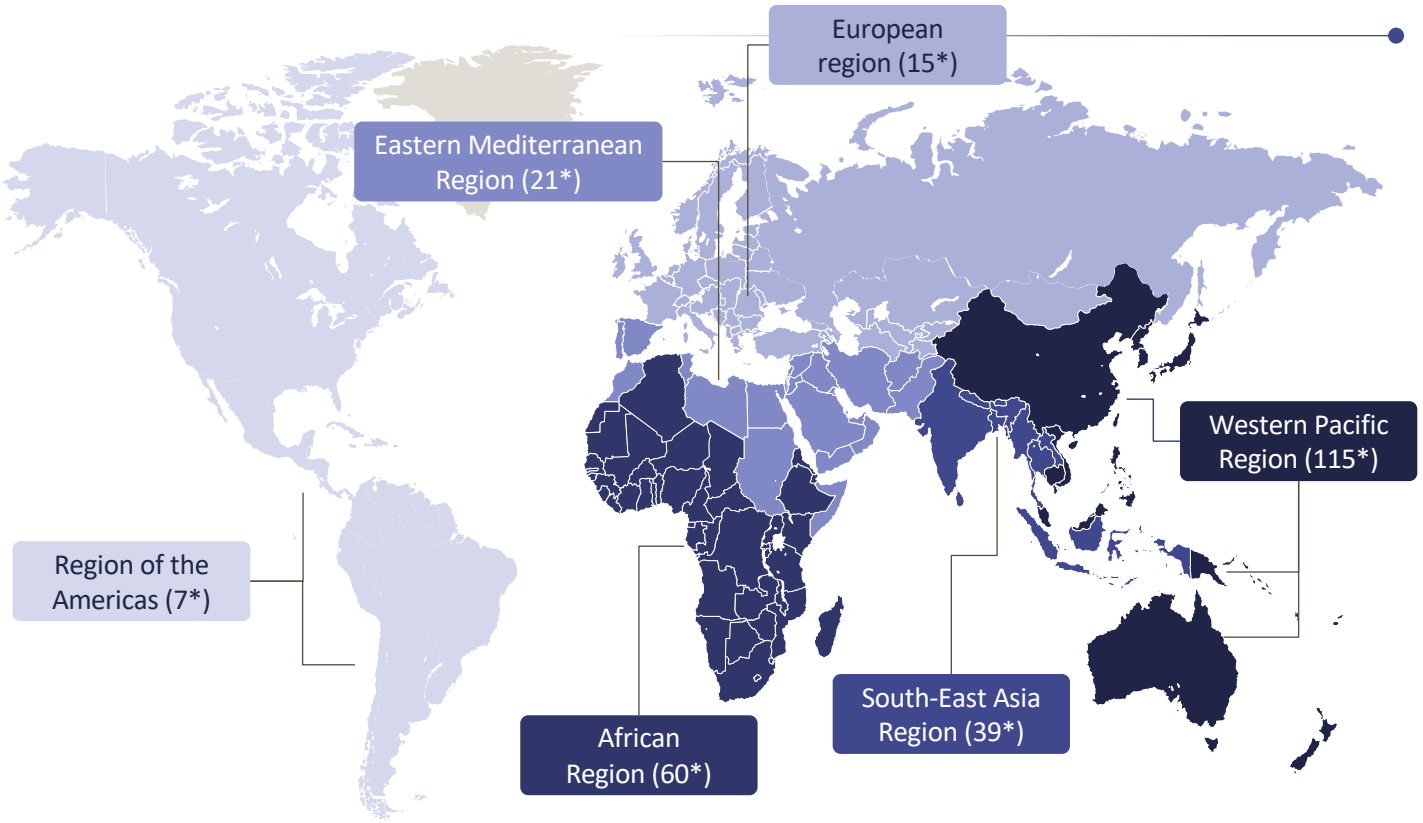
Epidemiology of Hepatitis B

Only 8% of patients diagnosed with Chronic Hepatitis B (CHB) get treated

Estimated prevalence



257 million people with CHB

22 million people get diagnosed

1.7 million people treated

*Estimated number of persons living with Hepatitis B Virus (millions) ; color gradient of the regions specify prevalence in increasing order
CHB = Chronic Hepatitis B
Global Hepatitis Report 2017. World Health Organization. https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/ Accessed on November 29, 2019

Thank you to Steve Hood at GSK for providing these slides

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A tale of 2 oligos - ASO to treat patients with CHB

Meet the Molecules

- **GSK3228836 (GSK836 aka ISIS 505358)** is a 20mer ASO gapmer targeting a shared region in all HBV mRNAs
  - The sequence is specific to HBV (no cross-reactivity with cellular mRNAs) and is highly conserved (>90%) across all HBV genotypes
  - GSK836 co-developed with Ionis since 2015 and in-licenced in 2019
- **GSK3389404 (GSK404)** is a GalNAc conjugated GSK836 designed to enhance hepatocyte uptake via ASGPR
  - Circulates as GSK404 in plasma but rapidly converted to GSK836 on uptake into the tissues
  - GSK404 was developed by GSK from 2015 and in-licenced in 2019

Non-clinical Package

- Efficacy in in vitro and in vivo HBV models
- Full pre-clin tox package (sc) in mouse and cyno + repro, safety pharm etc
- Sensitive LCMS assay developed to measure GSK404 & GSK836 in plasma and tissue

Clinical Package

- Ph1 study in Healthy Volunteers (UK & Canada)
- Ph2a Studies in Asian patients

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Bioanalytical challenges of 2 active molecules in plasma and tissue

Analytical Challenge

- **GSK836** is the major tissue metabolite of **GSK404** in pre-clin species
- **GSK404** is the major circulating molecule following SC dosing of **GSK404** in pre-clin and clinical plasma but some **GSK836** is released from the tissue over time
- GLP validated assay are required to support GLP tox

Available Assays (in 2015)

- Elisa based assays were gold standard (1ng/ml) but could not distinguish **GSK836** and **GSK404** (or their metabolites)
- UV-LC method used to quantify **GSK404** for CMC

Time for a new approach……

- Liq-liq extraction for tissue and plasma
- LCMS with internal standard
- GLP validated for tissue (50ng/g) and plasma (10 & 1ng/ml)

Thank you to Steve Hood at GSK for providing these slides GSK

Han *et al* (2019) Clin Pharm in Drug Dev 8 (6) 790-801

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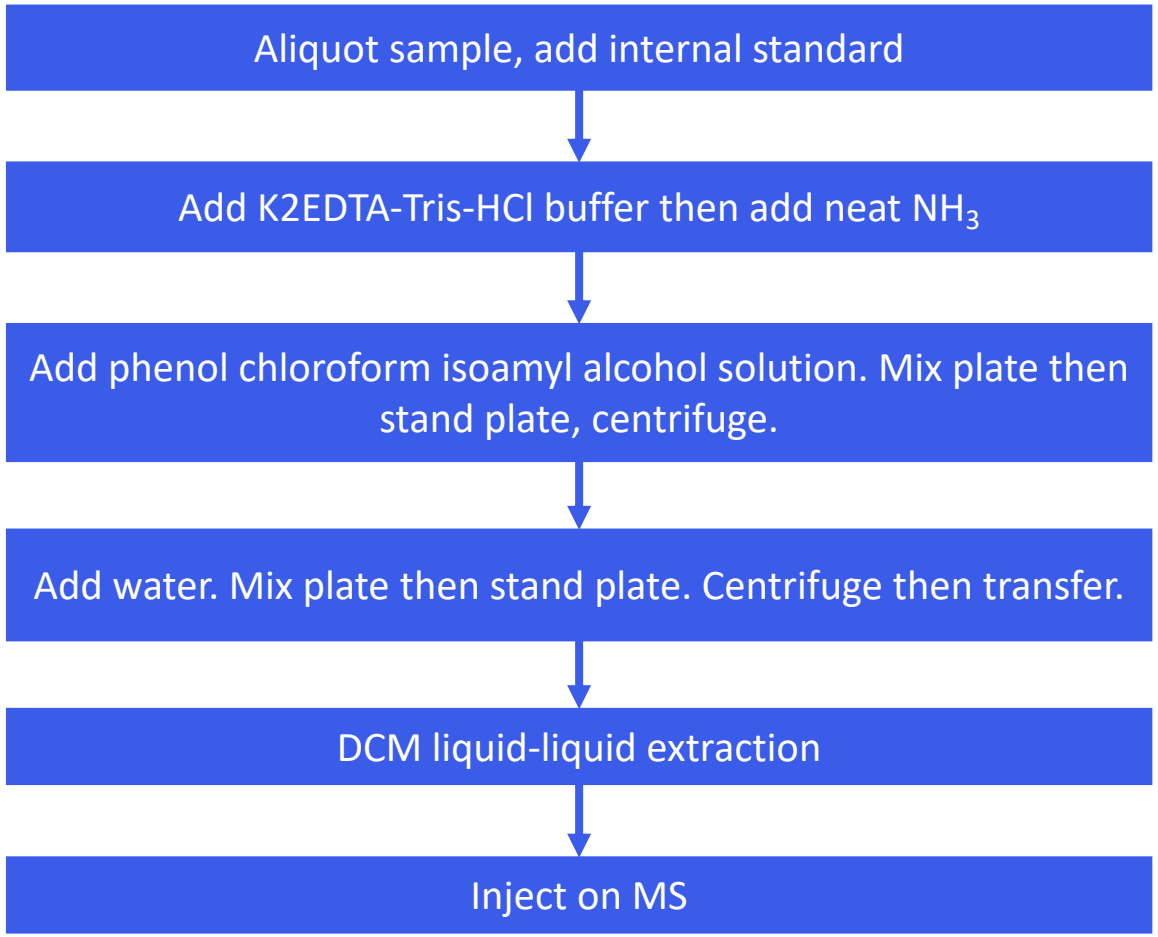
Transfer method from tandem lab in SLC:



Mobile Phase A and Mobile Phase B contained mixture of EDTA, DIPEA, TEA and HFIP. MPA was mainly aqueous, MPB was mix of MPA with 40% Acetonitrile

Sciex API 5000 with Shimadzu Nexera

Clarity 3 µm Oligo-RP 50 x 2 mm column used

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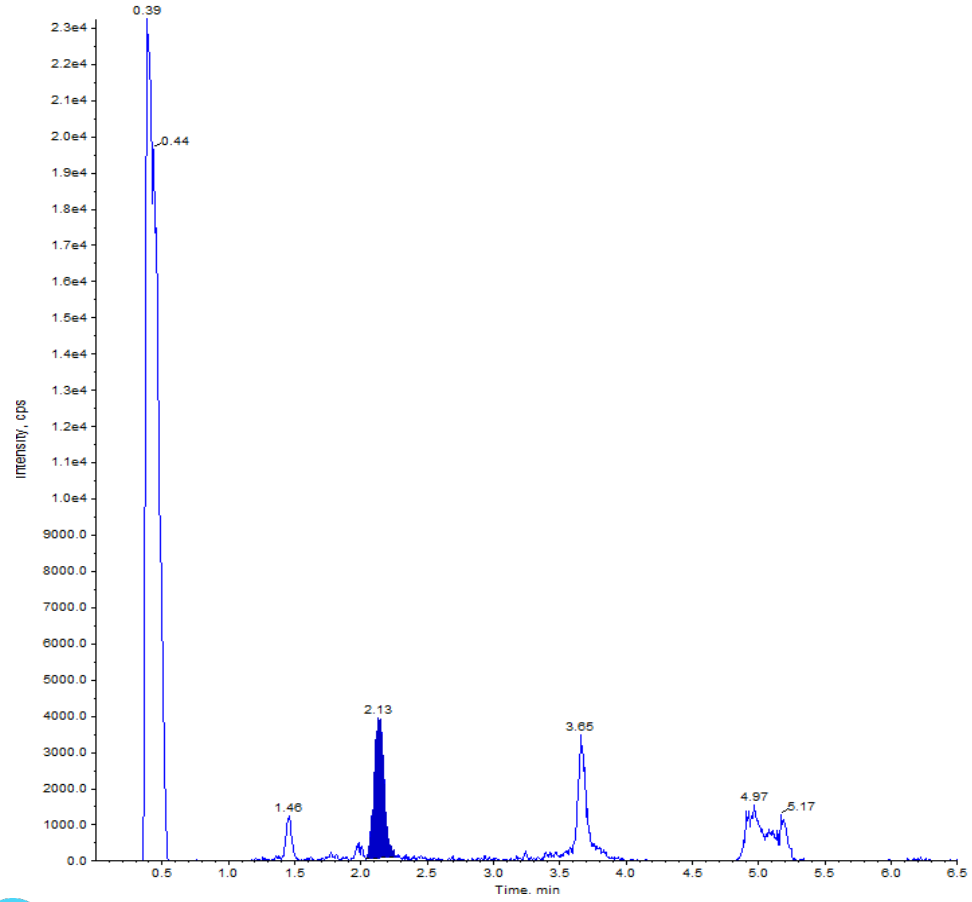
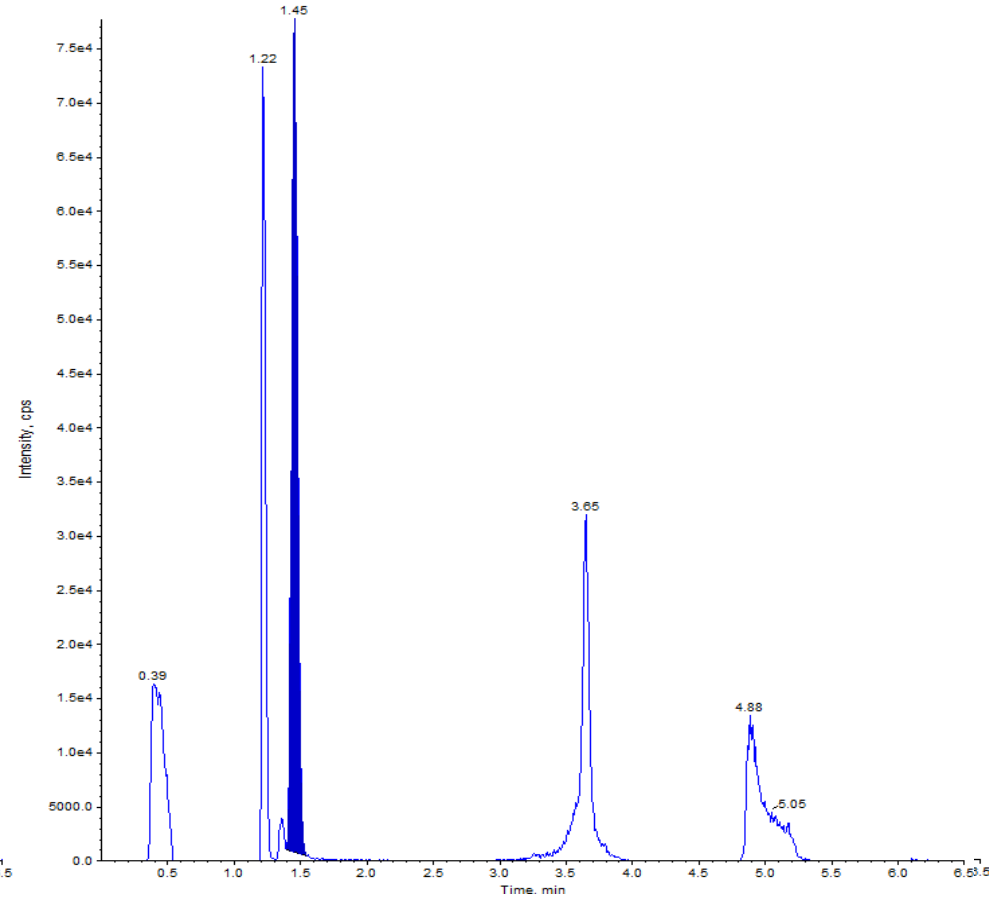
Pre-validation extraction results showed some issues:

- **Severe carryover – even with a false gradient!** → 100% of LLOQ in first injection which persisted. Low Cal & QC failure!
- **Deteriorating chromatography throughout batch**





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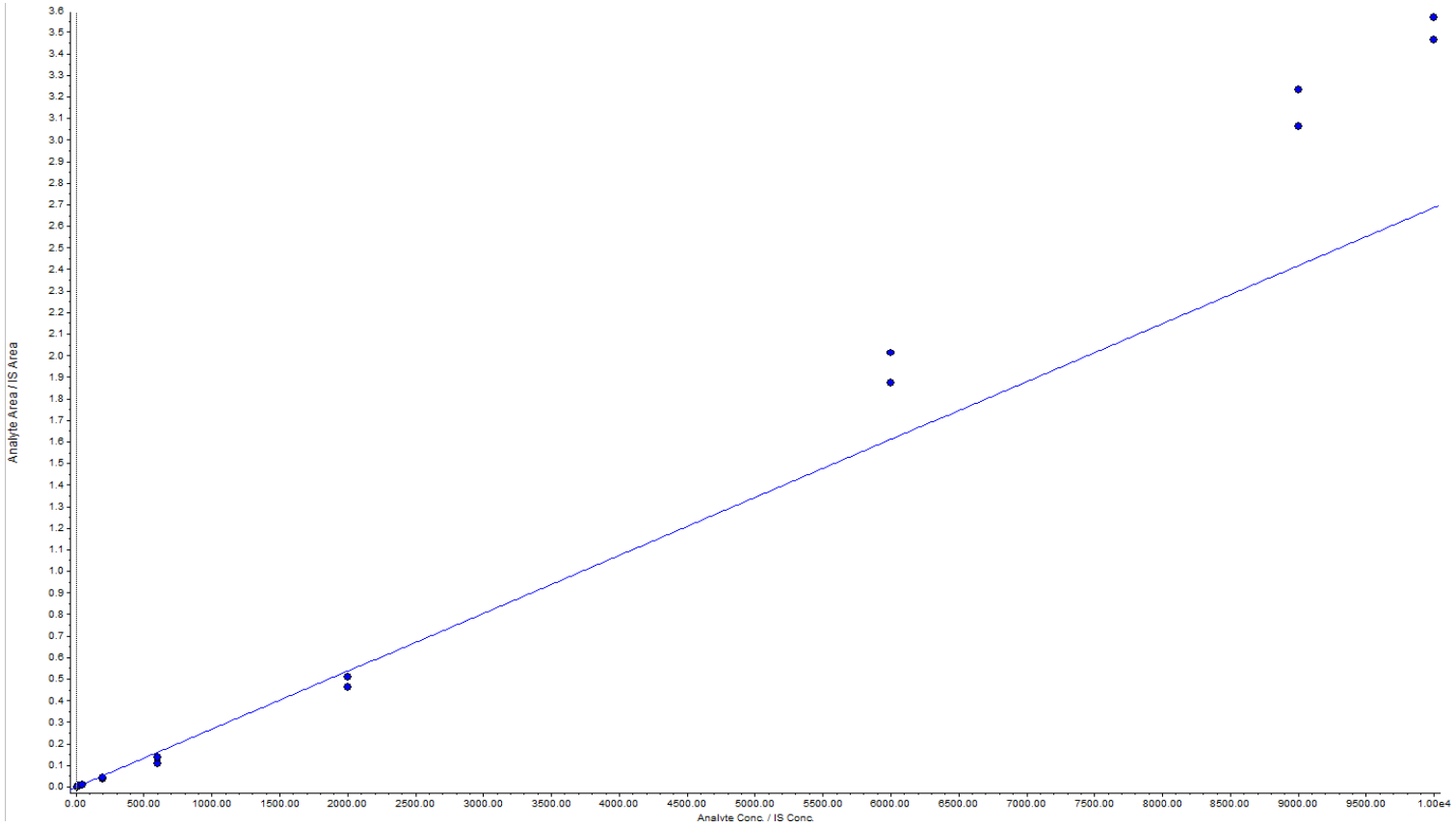
Monkey successfully validated. Also successfully managed to validate a human assay for both analytes and Shanghai validated a lower Human method for just GSK3228836.

Issues were seen when mouse, rat and rabbit went into validation:

- **Lack of linearity and variability throughout batches – especially at the lower end**
- **Response drift and poor column lifespan**



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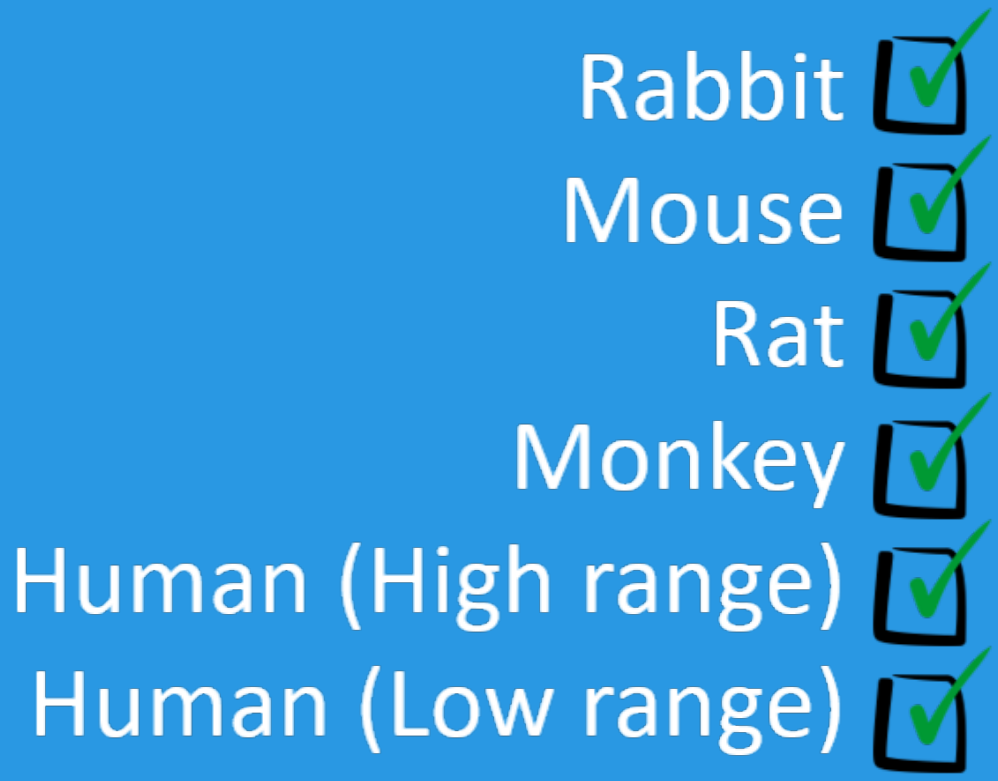
Success!



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Hepatitis B: can GSK's bepirovirsen deliver functional cure?

GSK's antisense oligonucleotide is looking to further prove its value in disrupting viral replication via a Phase III test.

GSK presents promising new data for bepirovirsen, an investigational treatment for chronic hepatitis B

Positive data for bepirovirsen from B-Clear phase IIb trial presented at American Association for the Study of Liver Diseases' Meeting with simultaneous publication in the New England Journal of Medicine

Ionis announces GSK has advanced bepirovirsen into Phase 3 development

February 1, 2023 at 9:05 AM EST

Efficacy and Safety of Bepirovirsen in Chronic Hepatitis B Infection

Man-Fung Yuen, M.D., Ph.D., D.Sc., Seng-Gee Lim, M.B., B.S., M.D., Robert Plesniak, M.D., Ph.D., Keiji Tsuji, M.D., Ph.D., Harry L.A. Janssen, M.D., Ph.D., Cristina Pojoga, M.D., Ph.D., Adrian Gadano, M.D., Ph.D., Corneliu P. Popescu, M.D., Ph.D., Tatyana Stepanova, M.Sc., Tarik Asselah, M.D., Ph.D., Gheorghe Diaconescu, M.D., Ph.D., Hyung Joon Yim, M.D., Ph.D., et al., for the B-Clear Study Group*

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Our previous experience at Labcorp:

- **DNA oligonucleotide extraction through SPE alone**

Bioanalysis. 2012 Jun;4(12):1457-69. doi: 10.4155/bio.12.117.

Development of a bioanalytical method for quantification of a 15-mer oligonucleotide at sub-ng/ml concentrations using LC-MS/MS

Martyn Hemsley [1], Matthew Ewles, Lee Goodwin

- **Oligonucleotide extraction using phenol-chloroform LLE and SPE**

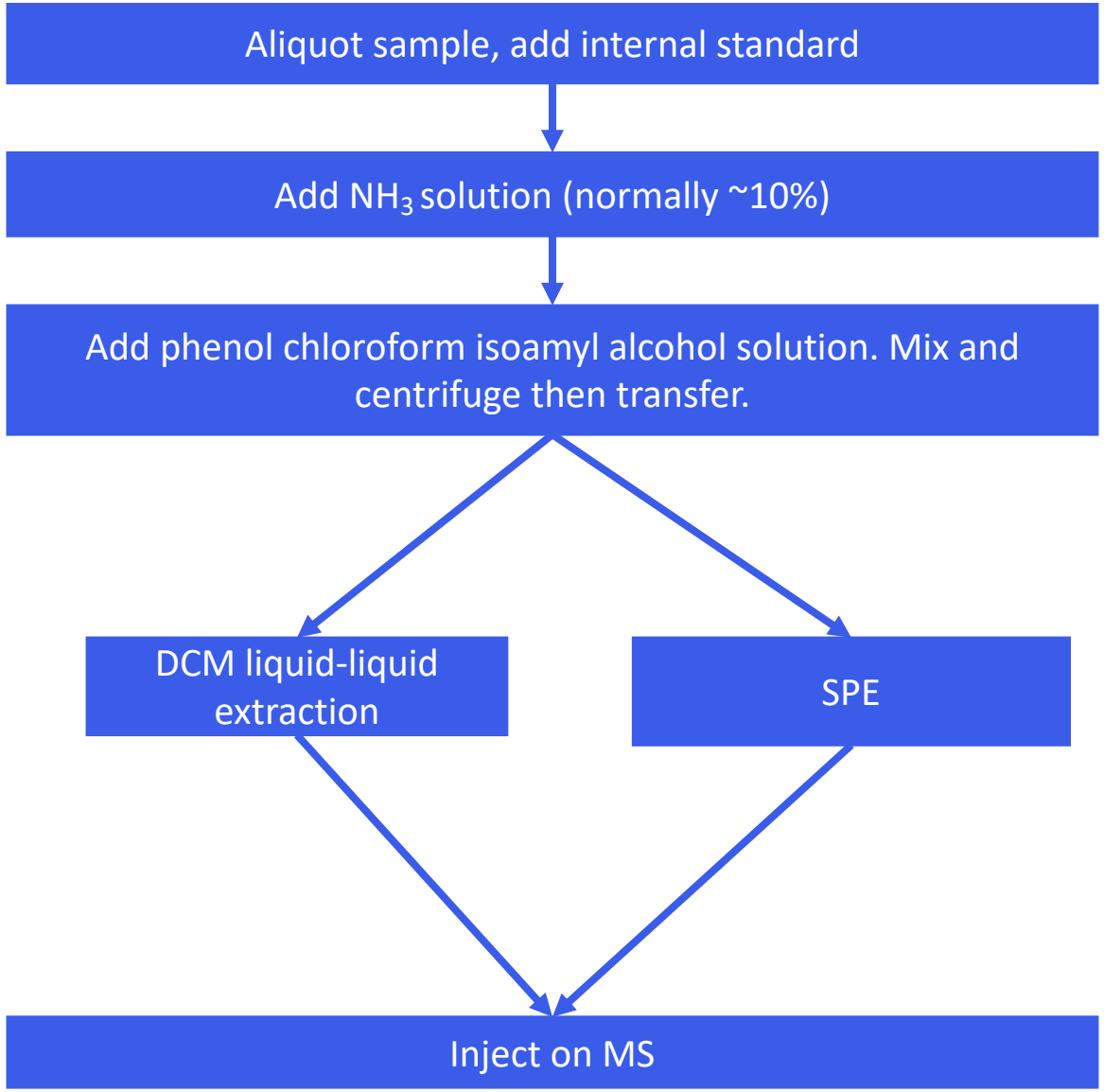
Bioanalysis. 2014 Feb;6(4):447-64. doi: 10.4155/bio.13.319.

Quantification of oligonucleotides by LC-MS/MS: the challenges of quantifying a phosphorothioate oligonucleotide and multiple metabolites

Matthew Ewles [1], Lee Goodwin, Anneliese Schneider, Tanja Rothhammer-Hampl

Refined previous method:

- Removal of unnecessary steps
- DIPEA removal
- Removal of K2 EDTA Tris-Buffer
- Removal of EDTA from mobile phases
- DCM or SPE
- Clarity OTX kits



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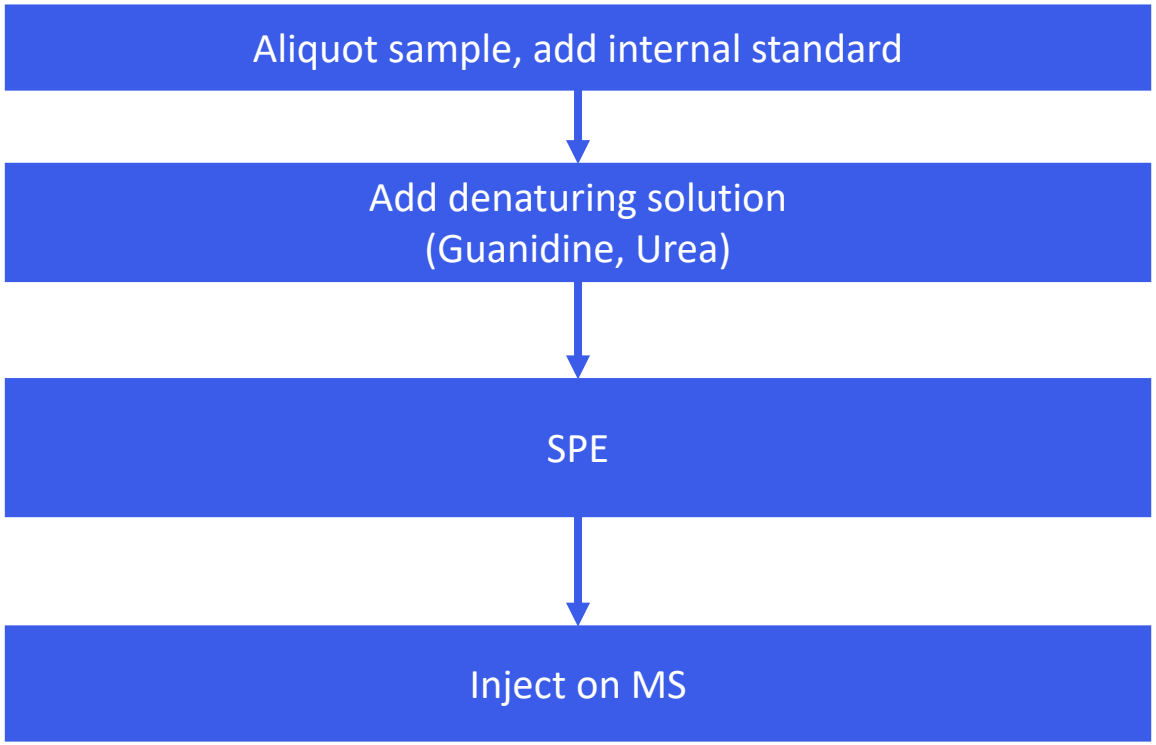
When phenol-chloroform doesn't work . . .

Fatty acid conjugated oligonucleotides



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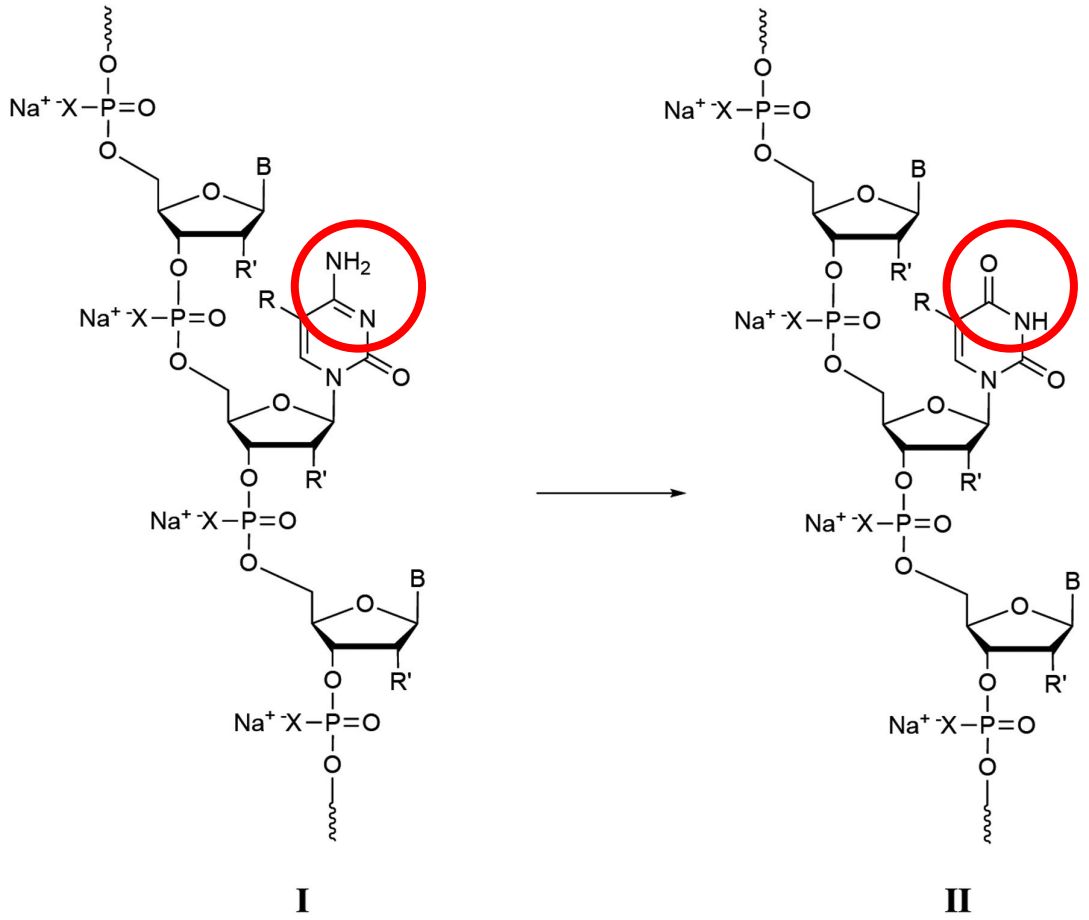
A New issue?

- **Deamination of oligonucleotides**



Rentel, Claus et al. "Determination of oligonucleotide deamination by high resolution mass spectrometry." *Journal of pharmaceutical and biomedical analysis* vol. 173 (2019): 56-61. doi:10.1016/j.jpba.2019.05.012

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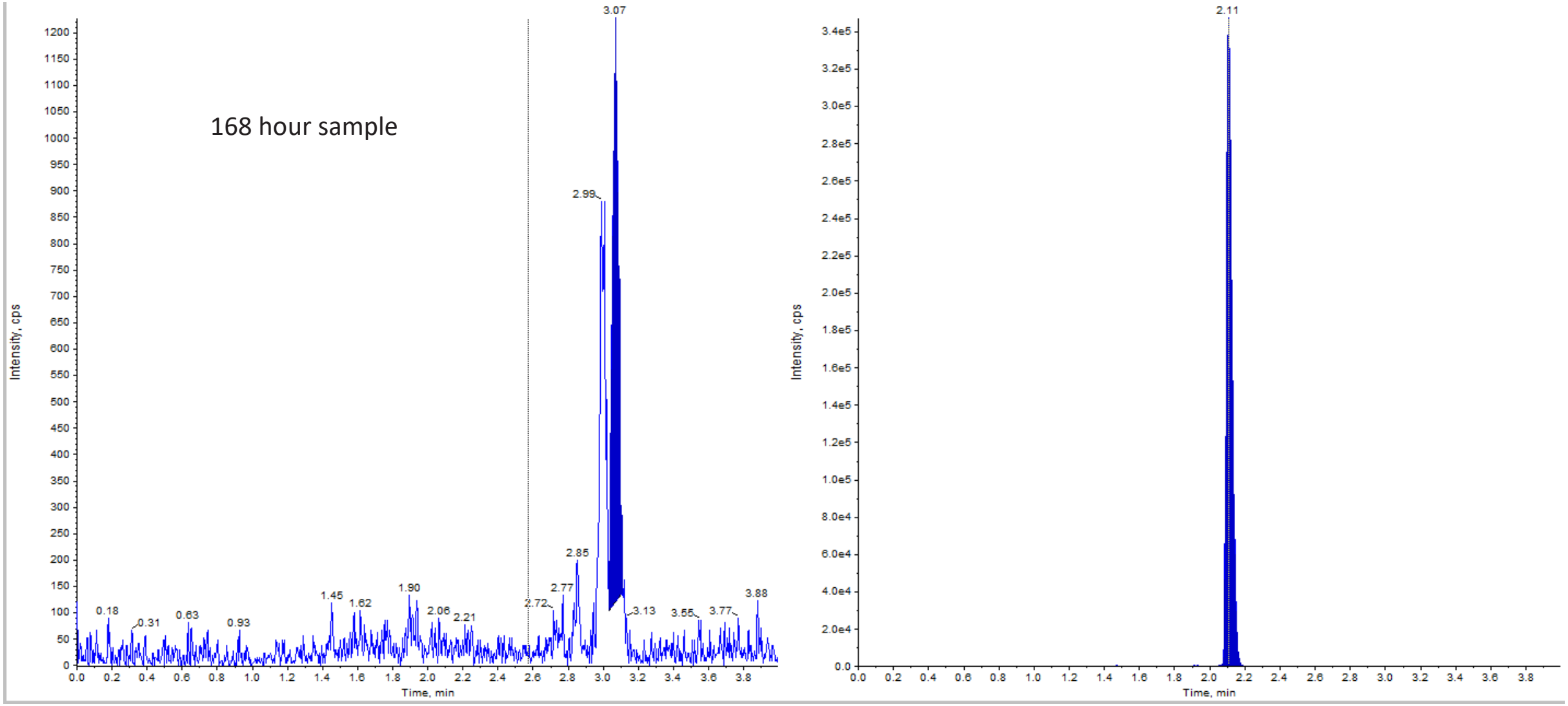
Chromatography in plasma:



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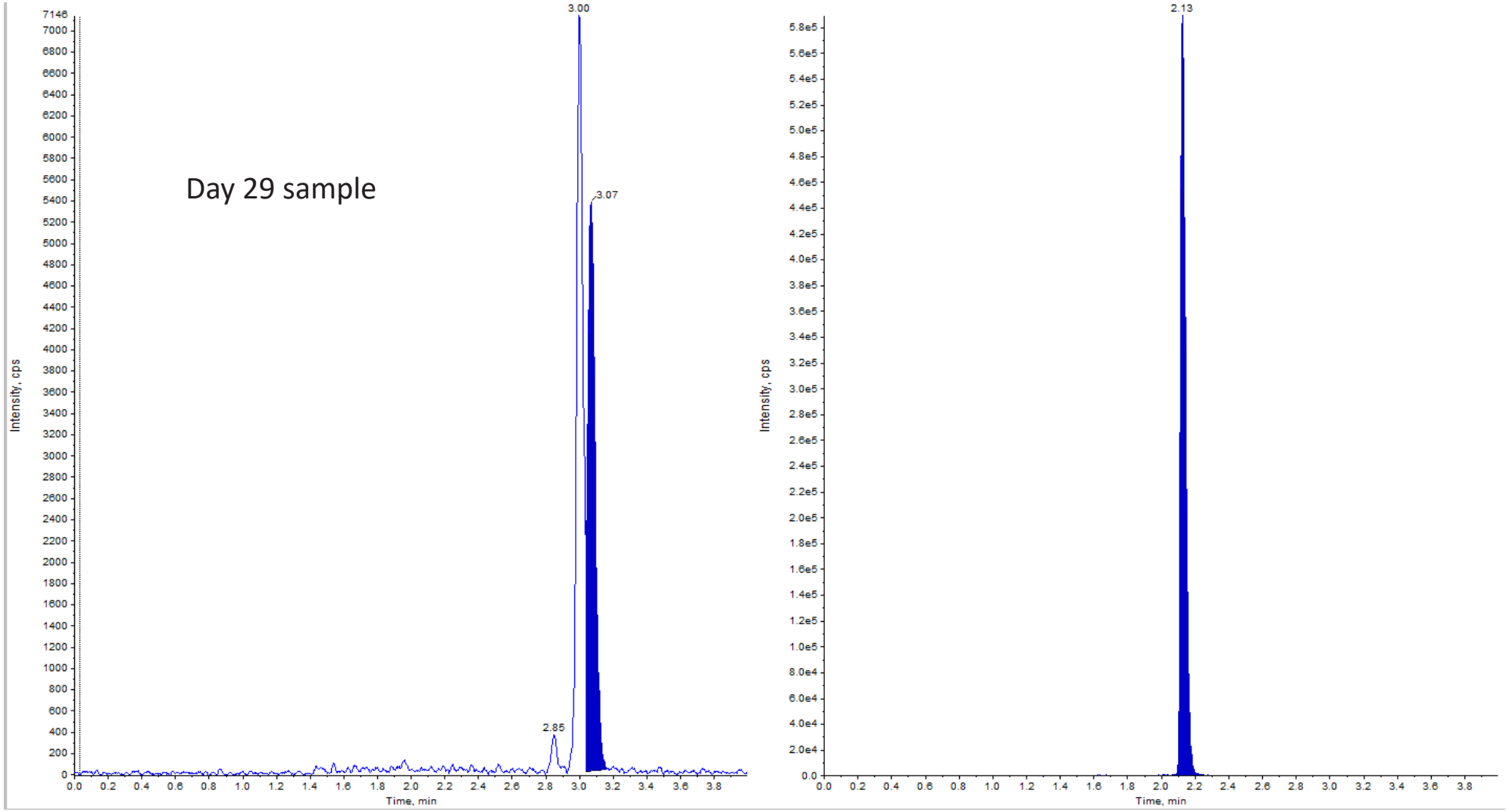
Chromatography in CSF:



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Thank you for listening!

Special thanks to:

Steve Hood, GSK

Matthew Ewles, Labcorp UK

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